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CONTENTS

REVIEW ARTICLES

- Cholesteryl Ester Transfer Protein: A Potential Target for the Treatment of Coronary Artery Disease**
HARSHA PATEL, JIGNA SHAH, SUNITA PATEL AND I. S. ANAND 735-740
- Properties and Formulation of Oral Drug Delivery Systems of Protein and Peptides**
A. SEMALTY, MONA SEMALTY, R. SINGH, S. K. SARAF AND SHUBHINI SARAF 741-747

RESEARCH PAPERS

- Fabrication and Evaluation of Asymmetric Membrane Osmotic Pump**
C. S. CHAUHAN, M. S. RANAWAT AND P. K. CHOUDHURY 748-752
- Studies of Disintegrant Properties of Seed Mucilage of *Ocimum gratissimum***
RAVIKUMAR, A. A. SHIRWAIKAR, ANNIE SHIRWAIKAR, S. LAKHSHMANA PRABU, R. MAHALAXMI, K. RAJENDRAN AND C. DINESH KUMAR 753-758
- Simultaneous Spectroscopic Estimation of Ezetimibe and Simvastatin in Tablet Dosage forms**
S. J. RAJPUT AND H. A. RAJ 759-762
- Formulation and Optimization of Carbamazepine Floating Tablets**
D. M. PATEL, N. M. PATEL, N. N. PANDYA AND P. D. JOGANI 763-767
- Effects of *Medicago sativa* on Nephropathy in Diabetic Rats**
M. S. MEHRANJANI, M. A. SHARIATZADEH, A. R. DESFULIAN, M. NOORI, M. H. ABNOSI AND Z. H. MOGHADAM 768-772
- Development of Hospital Formulary for a Tertiary Care Teaching Hospital in South India**
R. J. D'ALMEIDA, LEELAVATHI D. ACHARYA, PADMA G. M. RAO, J. JOSE AND RESHMA Y. BHAT 773-779
- Simultaneous Spectrophotometric Estimation of Rosiglitazone Maleate and Glimepiride in Tablet Dosage Forms**
ANJU GOYAL AND I. SINGHVI 780-783
- Preparation, Characterization and Antimicrobial Activity of Acrylate Copolymer Bound Amoxycillin**
J. S. PATEL, H. R. PATEL, N. K. PATEL AND D. MADAMWAR 784-790
- Haematitic Evaluation of *Lauha Bhasma* and *Mandura Bhasma* on HgCl₂-Induced Anemia in Rats**
P. K. SARKAR, P. K. PRAJAPATI, A. K. CHOUDHARY, V. J. SHUKLA AND B. RAVISHANKAR 791-795
- RPHPLC Method for the Estimation of Glibenclamide in Human Serum**
S. D. RAJENDRAN, B. K. PHILIP, R. GOPINATH AND B. SURESH 796-799
- 2D QSAR of Arylpiperazines as 5-HT_{1A} Receptor Agonists**
URMILA J. JOSHI, SONALI H. TIKHELE AND F. H. SHAH 800-804
- Antiproliferative and Cancer-chemopreventive Properties of Sulfated Glycosylated Extract Derived from *Leucaena leucocephala***
AMIRA M. GAMAL-ELDEEN, H. AMER, W. A. HELMY, H. M. RAGAB AND ROBA M. TALAAT 805-811

SHORT COMMUNICATIONS

- Simultaneous Derivative and Multi-Component Spectrophotometric Determination of Drotaverine Hydrochloride and Mefenamic Acid in Tablets**
P. P. DAHIVELKAR, V. K. MAHAJAN, S. B. BARI, A. A. SHIRKHEDKAR, R. A. FURSULE AND S. J. SURANA 812-814
- Design and Synthesis of Substituted 2-Naphthylxyethylamines as Potential 5-HT_{1A} Antagonists**
URMILA J. JOSHI, R. K. DUBE, F. H. SHAH AND S. R. NAIK 814-816
- Diuretic Activity of *Lagenaria siceraria* Fruit Extracts in Rats**
B. V. GHULE, M. H. GHANTE, P. G. YEOLE AND A. N. SAOJI 817-819
- Determination of Racecadotril by HPLC in Capsules**
S. L. PRABU, T. SINGH, A. JOSEPH, C. DINESH KUMAR AND A. SHIRWAIKAR 819-821
- Novel Spectrophotometric Estimation of Frusemide Using Hydrotropic Solubilization Phenomenon**
R. K. MAHESHWARI, S. DESWAL, D. TIWARI, N. ALI, B. POTHEH AND S. JAIN 822-824
- In Vivo* Pharmacokinetic Studies of Prodrugs of Ibuprofen**
ABHA DOSHI AND S. G. DESHPANDE 824-827
- Protective Effect of *Tamarindus indica* Linn Against Paracetamol-Induced Hepatotoxicity in Rats**
B. P. PIMPLE, P. V. KADAM, N. S. BADGUJAR, A. R. BAFNA AND M. J. PATIL 827-831
- Simultaneous Estimation of Atorvastatin Calcium and Amlodipine Besylate from Tablets**
P. MISHRA, ALKA GUPTA AND K. SHAH 831-833
- Development and Validation of a Simultaneous HPTLC Method for the Estimation of Olmesartan medoxomil and Hydrochlorothiazide in Tablet Dosage Form**
N. J. SHAH, B. N. SUHAGIA, R. R. SHAH AND N. M. PATEL 834-836
- Orodispersible Tablets of Meloxicam using Disintegrant Blends for Improved Efficacy**
P. V. SWAMY, S. H. AREEFULLA, S. B. SHIRSAND, SMITHA GANDRA AND B. PRASHANTH 836-840
- Spectrophotometric Method for Ondansetron Hydrochloride**
SRADHANJALI PATRA, A. A. CHOUDHURY, R. K. KAR AND B. B. BARIK 840-841
- HPTLC Determination of Artesunate as Bulk Drug and in Pharmaceutical Formulations**
S. P. AGARWAL, A. ALI AND SHIPRA AHUJA 841-844
- Simultaneous Spectrophotometric Estimation of Metformin and Repaglinide in a synthetic mixture**
J. R. PATEL, B. N. SUHAGIA AND B. H. PATEL 844-846
- Synthesis and Antiinflammatory Activity of Substituted (2-oxochromen-3-yl) benzamides**
V. MADDI, S. N. MAMLEDESAI, D. SATYANARAYANA AND S. SWAMY 847-849
- Evaluation of Hepatoprotective Activity of Ethanol Extract of *Prosopium acerifolium* Ster Leaves**
S. KHARPATE, G. VADNERKAR, DEEPTI JAIN AND S. JAIN 850-852
- New Antihistaminic Agents: Synthesis and Evaluation of H₁-Antihistaminic actions of 3-[(N,N-Dialkylamino)alkyl]-1,2,3,4-tetrahydro-(1H)-thioquinazolin-4(3H)-ones and Their oxo Analogues**
M. B. RAJU, S. D. SINGH, A. RAGHU RAM RAO AND K. S. RAJAN 853-856

Preparation, Characterization and Antimicrobial Activity of Acrylate Copolymer Bound Amoxicillin

J. S. PATEL, H. R. PATEL¹, N. K. PATEL AND D. MADAMWAR^{1*}

Department of Chemistry, ¹Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar - 388 120, India

Patel, *et al.*: Antimicrobial Activity of Acrylate Copolymer Bound Amoxicillin

Alternate copolymers of n-butyl methacrylate and maleic anhydride were prepared and characterized. Amoxicillin was bound on copolymers through pendent anhydride groups by chemical bonding. *In vitro* release rates were investigated in 0.1 M phosphate buffer solution with varying pH at 37° as well as in biological media. The release rates of the investigated model drug molecules followed first order kinetics and were dependent on buffer concentration and pH. It was observed that molecular weight of copolymers varied from 8000 to 22,000 g/mol and duration of the delay period prior to release increased from 6 to 9 d. P(BMA-MA-1) showed increase in cumulative release rate with increase in pH unit from 3.8 to 7.2, which further decreased from pH 7.8 and 8.0, respectively. With P(BMA-MA-2) gradual increase in release activity was observed in acidic range and in neutral to alkaline condition (pH 7.0 to 8.0) the release activity lasted for 7 to 9 d respectively. Similar condition for P(BMA-MA-3) showed cumulative release activity for 9 d in acidic pH, which further decreased to 7 d from pH 7.0 to 8.0. P(BMA-MA-1, 2 and 3) showed maximum inhibition of 40%, 59.3% and 72.8% towards *Bacillus subtilis*, 40%, 44% and 65.4% inhibition against *Staphylococcus aureus* and 41.2%, 51.7% and 69.1% inhibition against *Escherichia coli* at 48 h of incubation.

Key words: Acrylate polymers, maleic anhydride, copolymer, antimicrobial, amoxicillin

Vinyl polymers, methacrylate polymers, polyamides and poly(ethylene oxide) polymers have been widely studied as drug carriers due to their diversity, multi-functionality and biocompatible nature¹⁻⁶. They do not form toxic byproducts during their biodegradation and which have tendency to swell, when they come in contact with biological environment⁷. Amoxicillin possesses a broad antibacterial spectrum, but exerts short half-life values, which demand frequent drug administration. Therefore, continuous infusion has been suggested as the most beneficial mode of β -lactams⁸⁻¹⁰. Amoxicillin has been reported to be successfully used in various infections¹¹ including in septic absorptions, urinary tract infections, upper and lower respiratory infections, skin and soft tissue and GI tract infections.

The present studies describe controlled release of amoxicillin from the poly (butyl methacrylate-co-maleic anhydride)-amoxicillin [P(BMA-MA)AMOX] controlled-release system. In this system, the drug is covalently bound to polymeric matrix in the presence

of triethylamine (TEA) as a catalyst. This conjugate can be made to release drugs at controlled rates over extended periods of time. The specific goal of this investigation was to develop an improved oral dosage form for amoxicillin, a representative beta-lactam antibiotic.

MATERIALS AND METHODS

Amoxicillin was obtained from Vitara Chemicals Ltd., (Nandesari, Vadodara). Butylmethacrylate was obtained from Gujarat State Fertilizers Ltd., (Vadodara, Gujarat). Maleic anhydride (MA) and 2,2'-azobisisobutyronitrile were purchased from Fluka. Butylmethacrylate was purified using an inhibitor remover for hydroquinone and monomethylether. AIBN (azobisisobutyrylnitrile) was purified as described by Szajnecki *et al*¹². All other chemicals used were of analytical grade.

Synthesis and characterization of copolymers:

N-butylmethacrylate and maleic anhydride (at different molar ratios) along with AIBN was dissolved in distilled toluene. The solution was degassed by bubbling nitrogen gas for 10 min. Polymerization

*For correspondence

E-mail: datta_madamwar@yahoo.com

occurred as the solution was heated under constant stirring at 75° for 4 h. Polymers were recovered by precipitation in methanol (yield 80-85%). The homopolymer of BMA formed was removed from the copolymer by Soxhlet extraction (which is non-solvent for copolymer).

The average molecular weights were determined by gel permeation chromatography in THF. Monodispersed polystyrene standards were used for calibration. Copolymer composition was determined by estimating molar proportion of any one of the monomer in the copolymer. The MA content of the copolymer was determined by esterification-hydrolysis method¹³. Viscosity measurement of copolymers was carried out in dimethylformamide (DMF) at 30°. An Ubbelohde suspended level viscometer was used to measure viscosity¹⁴ by measuring the time (± 0.05 s) of flow of known amount of liquid (solvent and solution of different concentration) at constant temperature maintained in thermostat with accuracy of $\pm 0.1^\circ$.

Incorporation of amoxicillin:

The butylmethacrylate-maleic anhydride copolymer with different mol % of anhydride was ground to obtain 200 mesh cut. The copolymer was cycled through washes with methanol and cold distilled water to remove unreacted anhydride group¹⁵ and was dried at 40°. In the method, 1 g (constant in all synthesis) of butylmethacrylate-maleic anhydride copolymer was dissolved in dry N,N-dimethyl formamide (DMF, 25 ml) along with catalyst triethyl amine (0.3 ml). A calculated amount of amoxicillin in dry DMF (12.5 ml) was added drop wise at 20-25°. The reaction mixture was stirred for 1 h and for variable times, depending on the experimental program at room temperature. On finishing the coupling process, 250 ml cold distilled water with 0.3 ml hydrochloric acid was introduced in order to precipitate the reaction product and the mixture was stirred for half an hour before filtration. The precipitates were filtered off, washed twice with distilled water. The polymer

bound amoxicillin samples were treated with (0.1 M) aqueous NaHCO₃ solution for the hydrolysis of unreacted anhydride group. The washed precipitate was collected and dried over calcium chloride in desiccator. Similar procedure was followed for coupling of amoxicillin on copolymers P(BMA-MA-2) and P(BMA-MA-3) respectively. All the washings were collected quantitatively. The amount of amoxicillin in the entire washed material was estimated colorimetrically using p-dimethyl aminobenzaldehyde reagent at 410 nm using HP-8452 Diode Array UV/Vis spectrophotometer as described by Sethi¹⁶⁻¹⁷. The results are mentioned in Table 1. Similar method was followed for copolymer 2 and 3, respectively. The concentration of bound amoxicillin was calculated by considering the initial amoxicillin taken for binding and the amount released in subsequent washings as unbound amoxicillin.

In Vitro drug release study:

In an experiment, 25 mg of polymer bound amoxicillin sample was suspended with 5 ml of the 0.1 M buffer (Tris-pH 3.8, citrate-pH 4.6, 5.8 and phosphate-pH 7.0, 7.2, 8.0) of varying pH as described for a fixed time of 24 h in triplicate. The released amoxicillin in buffer was separated by centrifugation at 3000 g for 15 min and amount of amoxicillin in supernatant, was determined spectrophotometrically as described above. The residues after centrifugation were again equilibrated with a fresh 5 ml portion of buffer. The process was repeated after every 24 h until no more elution of amoxicillin in the supernatant was observed. This method was adopted for all the three systems. The data was subjected to analysis by calculating the fractional release at different intervals of time (M_t/M_∞) and plotting the graph of these values against time, square root of time and log of time. From the linearity of the graph, the order of drug release was ascertained. The rate of release was obtained from the graph and correlated with the physicochemical characteristics of polymer and drug.

TABLE 1: CHARACTERIZATION OF N-BUTYL METHACRYLATE-MALEIC ANHYDRIDE COPOLYMER

Polymer system	Mole fraction in feed		Mole fraction in copolymer		Number average molecular weight (g/mol) Mn ^c x 10 ³	Weight average Molecular weight (g/mol) Mw ^c x 10 ³	Poly dispersity	Content of BAC in mg/g of copolymer	Intrinsic Viscosity (IV) (dl/g)
	M ₁	M ₂	m ₁	m ₂					
P (BMA-MA-1)	0.676	0.676	0.713	0.287	4.93	22.6	4.60	246	0.187
P (BMA-MA-2)	0.406	0.406	0.589	0.411	4.03	16.5	4.08	418	0.168
P (BMA-MA-3)	0.186	0.186	0.512	0.488	3.04	8.1	2.67	618	0.121

Where, M₁ = mole fraction of BMA in feed, m₁ = mole fraction of BMA in copolymer, M₂ = mole fraction of MA in Feed, m₂ = mole fraction of MA in copolymer

Antimicrobial activity:

The antimicrobial activity was studied on both gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli*) bacteria. The cultures were inoculated in nutrient broth and incubated for 12 h at 37°. Optical density of all the three cultures was adjusted to 0.3 by diluting with sterile nutrient broth. For antibacterial activity 50 ml of individual liquid culture in 250 ml flask with an initial O.D.₆₆₀ of 0.3 was spiked with 25 mg of prodrug and increase in growth (absorbance at 660 nm) was monitored at every 8 hours interval up to 48 h.

Statistical analysis:

Values reported are averages of the release activity data in triplicate. The Microsoft Excel (Release 2000) under Windows 98 operating system (ICON Software Technologies, Baroda, Gujarat, India) was used to carry out the statistical calculations. The values represented in the tables are the limits (3 SD Limits) within which the observed data lies. The ± 3 SD from the mean covers 95% of the observed data. The observed mean effect is significant with a *P* value less than 0.035.

RESULTS AND DISCUSSION

Copolymers of n-butyl methacrylate and maleic anhydride comparatively of low average molecular weight (8113 to 22638) was prepared by solution polymerization technique using AIBN as initiator and characterized by viscosity to use as a drug carrier, the results are described in Table 1. Weight average molecular weights were 8113 to 22638 g/mol with a poly-dispersity index (Mw/Mn) of 2.67 to 4.60. The feed proportions of each co-monomer are mentioned in Table 1, with the corresponding alternate copolymers referred to as P(BMA-MA-1), P(BMA-MA-2) and P(BMA-MA-3), respectively. MA contents determined by titration were 0.28, 0.41 and 0.48 mole % for the three copolymers. Reactivity ratio of copolymer of BMA and MA was determined by using linear graphical method of Finemann and Ross (data not shown). The reactive ratio observed was 0.83 and 0.04, respectively.

Amoxicillin was bound with each of these three copolymers and the study of sustained release of the drug from each system was investigated by monitoring released amoxicillin in buffer by

spectrophotometric estimation method described under materials and methods. Coupling of the amoxicillin to the copolymer carriers in the presence of a TEA was carried out (Table 1). Amongst the solvents, DMF was found to be more suitable as amount of amoxicillin, coupled was about 80-85% compared to amount of ~45-50% in other solvent. To determine minimum time for maximum loading of drug the reactions were carried for various times (fig. 1). It indicates that maximum coupling accomplished at room temperature at constant stirring nearly with in 20 h indicated by minimum and constant acid value. Similarly to optimize mole ratio of anhydride, biological active compound and catalyst the coupling reaction, was carried out by varying mole ratios. The results given in fig. 2 reveals that amount of amoxicillin coupled to polymer increases with increase in anhydride content. Data in fig. 2 also reveals that the maximum loading of amoxicillin is 1 g to 1 g because the sites

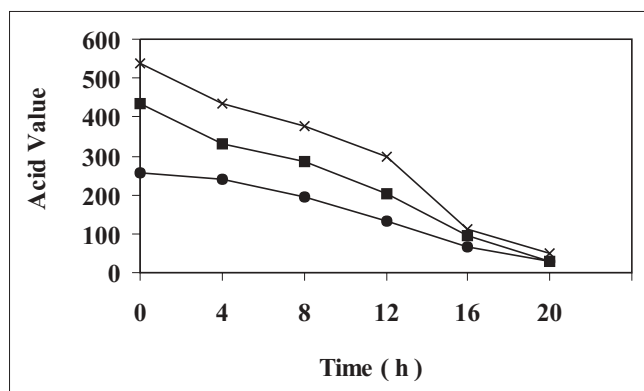


Fig. 1: Course of preparation of polymer bound amoxicillin (—●—) P(BMA-MA-1) AMOX, (—■—) P(BMA-MA-2) AMOX, (—×—) P(BMA-MA-3) AMOX

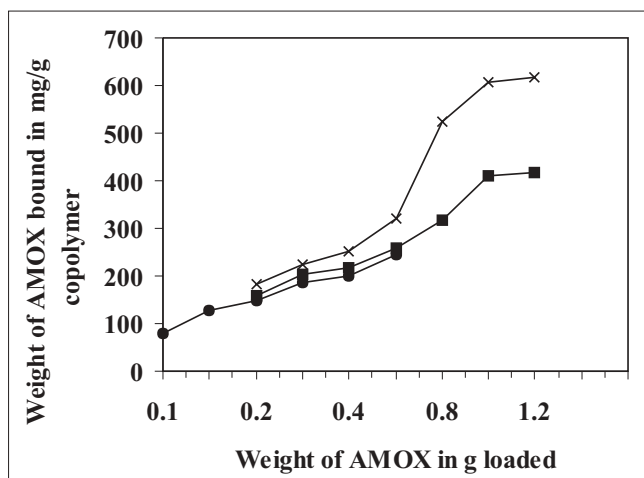


Fig. 2: Incorporation of amoxicillin per gram of copolymer (—●—) P(BMA-MA-1) AMOX, (—■—) P(BMA-MA-2) AMOX, (—×—) P(BMA-MA-3) AMOX

for coupling (anhydride) are not available. Thus, amount is leveled off beyond this point. The amount of amoxicillin covalently bound to copolymer was determined spectroscopically at 410 nm. Amoxicillin (24.6 mg), (41.8 mg) and (61.8 mg) was bound respectively to 100 mg each of copolymer P(BMA-MA-1), P(BMA-MA-2) and P(BMA-MA-3) in 20 h (Table 1).

The content of amoxicillin in the copolymer was determined via their characteristic UV absorption. After proper adjustment of concentration of amoxicillin, UV absorptions measurement were carried out at 286 nm (fig. 3). It was observed that copolymer does not absorb in this range and hence it did not interfere with the absorption of drug. The measurements were carried out using aqueous buffer solution of pH 7.8. Similar observation, were also noticed by Azori *et al*¹⁸ who worked on the model drug p-nitroaniline bound to polyanionic carrier.

Amoxicillin covalently bound to polymeric matrix P(BMA-MA) represents insoluble inert matrix. These matrices act as a retardant either by forming insoluble matrix or by formation of a skeleton. The rate determining step in controlling release rate from this formulation is the diffusion of liquid into the matrix. Drug release is triggered by penetration of eluting media into the matrices thereby releasing drug by hydrolysis, which is channelised by diffusion. The depletion zone gradually extends into the core of the matrix. In the absence of additive, drug release is prolonged and nonlinear. Plot of fractional drug release M_t/M_∞ against time, square root of time and log of time are shown in fig. 4, for the system

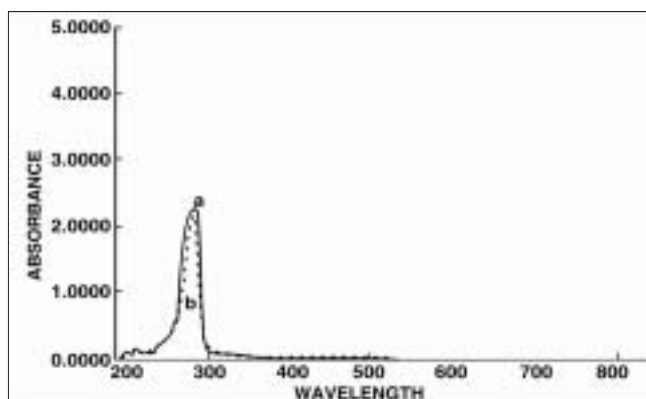


Fig. 3: Absorption Spectra of Amoxicillin in 0.1 M phosphate buffer at pH 7.8

Absorption spectra of (a) amoxicillin $C=1.0$ mg/ml (b) amoxicillin covalently bound to poly(butyl methacrylate-co-maleic anhydride) $C=1.1$ mg/ml in phosphate buffer at pH 7.8

P(BMA-MA-2)AMOX. From the plot it is clear that the systems behave as described above with the release profile of M_t/M_∞ vs. Square root of time being linear. Thus, these systems can be classified as mentioned above i.e. insoluble, inert matrices. Similar plot of the other polymer system can be expected to yield similar results.

The release activity of the drug depends on the, kind of polymeric system and the characteristics of the drug (molecular weight, solubility) and in every case the amount of drug release gradually decreases with time. Release activity studies for all the three polymeric prodrugs, was also studied at different pH taking into consideration the pH existing in digestive and circulatory systems. The experimental sets were kept in triplicate for each of the polymeric systems and incubated at 37° taking into consideration the normal body temperature.

We studied the effect of ionic strength on release activity of amoxicillin results indicates that, increasing the ionic strength of buffer medium decreases the rate of release activity (data not shown). It was observed that at buffer concentration of 0.1 M the drug was totally released in 9 and 7 d, respectively. Observation explains that during drug release equivalent acid groups are formed which can be compensated easily by higher concentration of buffering ion which in turn

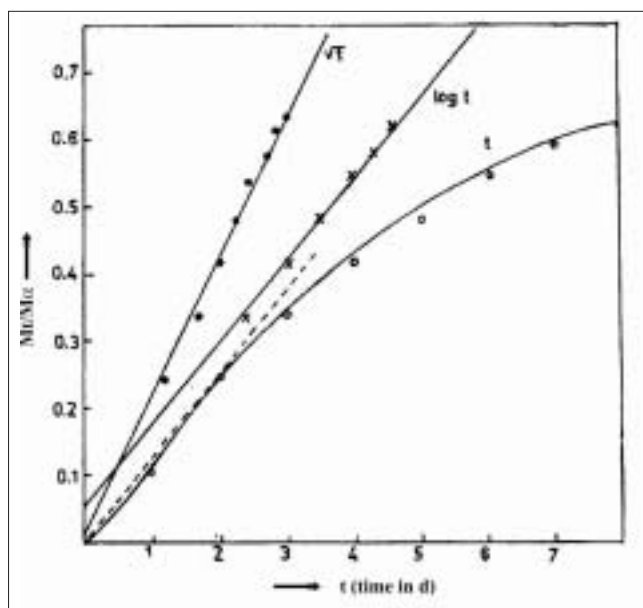


Fig. 4: Fractional drug release against t , \sqrt{t} and $\log t$ for P(BMA-MA-2)AMOX

Plot of fractional drug release against time, square root of time and log of time for P(BMA-MA-2)AMOX. t (—○—), \sqrt{t} (—●—) and $\log t$ (—×—)

favors easy and higher rate of release in comparison to lower buffering ion concentration.

Further experiments were conducted in 0.1 M concentration of citrate buffer (pH 3.8, 4.6 and 5.8) and phosphate buffer (pH 7.0, 7.2 and 8.0). Results revealed that P(BMA-MA-1) showed cumulative release of 48%, which lasted for maximum 8 d in acidic pH and in alkaline range 53% of cumulative release was observed which lasted for 9 d (Table 2). It was observed that on increasing the payload of amoxicillin i.e. in P(BMA-MA-2), 50-55% cumulative release was observed in acidic pH and 60-65% in alkaline pH. The release activity lasted for 9 d with this polymeric prodrug system (Table 3).

With P(BMA-MA-3) the release activity lasted for 9 d in acidic range with maximum cumulative release

of 60%, where as in alkaline range 80% cumulative release was observed which lasted for maximum 7 d (Table 4). From the results it can be concluded that increasing percentage of maleic anhydride increases drug release. Strong swelling was observed during the release of the drug from the copolymer in case of P(BMA-MA-2) and P(BMA-MA-3) after 2 d. The high rate of swelling was due to high carboxylic content, which also increased drug release. Increase in pH also increased the release activity.

Antimicrobial activity of amoxicillin in free and polymer bound form was determined. Experiments on antimicrobial activity of polymer bound amoxicillin samples were conducted in nutrient broth. To determine antimicrobial activity, optical density was measured as a function of time in each case. It is known that as bacterial growth increases optical

TABLE 2: *IN VITRO* RELEASE OF AMOXYCILLIN FROM P (BMA-MA-1) AMOX PREPARATION

Days (d)	pH 3.8 % Cumulative release	pH 4.6 % Cumulative release	pH 5.8 % Cumulative release	pH 7.0 % Cumulative release	pH 7.2 % Cumulative release	pH 8.0 % Cumulative release
1	7.75±0.01	5.60±0.01	15.50±0.02	12.16±0.01	13.27±0.15	14.37±0.10
2	17.69±0.01	7.75±0.01	18.80±0.01	23.21±0.01	22.66±0.15	15.48±0.10
3	18.80±0.02	12.74±0.03	27.14±0.15	30.40±0.02	30.96±0.03	30.96±0.15
4	19.35±0.01	16.61±0.03	36.72±0.02	31.51±0.02	38.21±0.01	37.59±0.10
5	21.56±0.03	21.60±0.12	38.12±0.02	33.72±0.15	40.91±0.01	46.99±0.10
6	24.89±0.10	28.24±0.15	43.54±0.01	39.25±0.01	46.99±0.02	49.76±0.20
7	-	33.25±0.15	47.12±0.15	42.01±0.15	49.20±0.02	53.07±0.20
8	-	36.10±0.20	47.89±0.04	45.88±0.01	51.31±0.10	-
9	-	-	-	46.99±0.03	51.86±0.10	-

P (BMA-MA-1) containing 6.14 mg of Amoxicillin in 25 mg of copolymer at temperature 37[±] 0.5^o.

TABLE 3: *IN VITRO* RELEASE OF AMOXYCILLIN FROM P (BMA-MA-2) AMOX PREPARATION

Days (d)	pH 3.8 % Cumulative release	pH 4.6 % Cumulative release	pH 5.8 % Cumulative release	pH 7.0 % Cumulative release	pH 7.2 % Cumulative release	pH 8.0 % Cumulative release
1	6.50±0.01	9.40±0.22	11.06±0.01	11.06±0.01	9.76±0.00	9.76±0.01
2	12.36±0.11	13.99±0.21	19.52±0.01	20.82±0.01	20.17±0.10	18.22±0.01
3	15.62±0.15	16.27±0.02	26.68±0.02	31.23±0.17	27.98±0.10	25.38±0.03
4	16.92±0.02	18.88±0.02	33.19±0.01	40.99±0.11	37.52±0.15	33.84±0.02
5	18.87±0.02	22.89±0.15	40.32±0.15	50.11±0.21	43.97±0.02	40.34±0.02
6	19.52±0.01	25.70±0.16	46.53±0.02	54.01±0.21	47.22±0.03	48.48±0.16
7	-	27.33±0.11	51.41±0.02	55.12±0.02	48.20±0.11	52.71±0.15
8	-	-	54.33±0.15	-	-	57.92±0.15
9	-	-	55.64±0.15	-	-	61.82±0.01

P (BMA-MA-2) containing 10.45 mg of Amoxicillin in 25 mg of copolymer at temperature 37[±] 0.5^o.

TABLE 4: *IN VITRO* RELEASE OF AMOXYCILLIN FROM P (BMA-MA-3) AMOX PREPARATION

Days (d)	pH 3.8 % Cumulative release	pH 4.6 % Cumulative release	pH 5.8 % Cumulative release	pH 7.0 % Cumulative release	pH 7.2 % Cumulative release	pH 8.0 % Cumulative release
1	6.20±0.11	15.41±0.10	16.51±0.00	14.74±0.03	26.63±0.22	28.52±0.23
2	12.76±0.15	39.83±0.10	32.57±0.10	31.47±0.01	43.79±0.04	56.80±0.11
3	18.05±0.10	56.12±0.15	34.55±0.04	47.53±0.01	60.52±0.04	76.95±0.10
4	22.45±0.10	59.86±0.05	40.71±0.04	59.19±0.02	73.28±0.12	88.95±0.10
5	24.43±0.05	60.96±0.05	48.19±0.01	66.24±0.03	76.58±0.12	91.25±0.03
6	29.71±0.02	61.18±0.22	53.92±0.01	72.40±0.03	78.34±0.03	93.26±0.01
7	33.33±0.16	61.62±0.05	58.54±0.01	73.50±0.03	80.54±0.15	-
8	35.41±0.01	63.81±0.05	60.74±0.02	-	-	-
9	-	-	60.96±0.02	-	-	-

P (BMA-MA-3) containing 15.45 mg of Amoxicillin in 25 mg of copolymer at temperature 37[±] 0.5^o.

TABLE 5: ANTIMICROBIAL ACTIVITY OF POLYMER BOUND AMOXYCILLIN AS A FUNCTION OF TIME AND INCREASING MA CONTENT AGAINST *B. SUBTILIS*

Time (h)	Control O.D.		Copoly-mer O.D.		P (BMA-MA-1) AMOX		P (BMA-MA-2) AMOX		P (BMA-MA-3) AMOX		Amoxycillin	
	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh
0	0.0060±0.00	-	0.0050±0.00	-	0.0050±0.00	-	0.0060±0.00	-	0.0020±0.00	-	0.0026±0.0002	98.2±0.3
8	0.1440±0.0020	10.6±0.1	0.1438±0.005	10.6±0.1	0.1300±0.0015	9.7±0.1	0.0970±0.0010	32.7±0.10	0.0026±0.0002	-	0.0069±0.0005	97.4±0.7
16	0.2640±0.0010	16.7±0.2	0.2642±0.0015	16.7±0.2	0.2120±0.0020	19.7±0.1	0.1730±0.0010	34.5±0.20	0.0069±0.0005	-	0.0154±0.0001	97.7±0.5
24	0.6680±0.0030	26.6±0.1	0.6684±0.0015	26.6±0.1	0.4670±0.0015	30.1±0.1	0.2550±0.0010	61.8±0.20	0.0154±0.0001	-	0.0188±0.001	97.9±0.5
32	0.8970±0.002	32.5±0.1	0.8975±0.0017	32.5±0.1	0.5040±0.0010	43.8±0.2	0.2890±0.0025	67.8±0.14	0.0188±0.001	-	0.0310±0.0015	97.5±0.2
40	1.2410±0.002	40.1±0.5	1.2409±0.002	40.1±0.5	0.7992±0.0012	54.1±0.2	0.3810±0.0020	69.3±0.10	0.0310±0.0015	-	0.0296±0.0012	97.9±0.2
48	1.4110±0.001	48.1±0.5	1.4114±0.002	48.1±0.5	0.8452±0.002	59.3±0.1	0.3840±0.0010	72.8±0.15	0.0296±0.0012	-	-	-

% Inh = Inhibition; O.D. = Optical density at 660 nm

TABLE 6: ANTIMICROBIAL ACTIVITY OF POLYMER BOUND AMOXYCILLIN AS A FUNCTION OF TIME AND INCREASING MA CONTENT AGAINST *E. COLI*

Time (h)	Control O.D.		Copoly-mer O.D.		P (BMA-MA-1) AMOX		P (BMA-MA-2) AMOX		P (BMA-MA-3) AMOX		Amoxycillin	
	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh
0	0.0040±0.00	-	0.0030±0.000	-	0.0040±0.00	-	0.0040±0.00	-	0.0020±0.00	-	0.0020±0.00	-
8	0.8770±0.002	8.7±0.2	0.8774±0.006	8.7±0.2	0.686±0.003	21.8±0.15	0.4410±0.025	49.7±0.4	0.0161±0.002	-	0.0161±0.002	98.2±0.5
16	1.2000±0.015	16.8±0.4	1.2100±0.005	16.8±0.4	0.818±0.004	31.8±0.20	0.5981±0.021	50.2±0.2	0.0410±0.001	-	0.0410±0.001	96.6±0.2
24	1.3510±0.020	32.9±0.3	1.3509±0.005	32.9±0.3	0.906±0.002	39.3±0.01	0.6152±0.030	54.5±0.2	0.0337±0.002	-	0.0337±0.002	97.5±0.15
32	1.5400±0.020	35.9±0.5	1.5411±0.010	35.9±0.5	0.8360±0.003	45.9±0.03	0.6312±0.005	59.1±0.5	0.0389±0.005	-	0.0389±0.005	97.5±0.15
40	1.6200±0.011	36.8±0.4	1.6212±0.010	36.8±0.4	0.8410±0.0015	48.1±0.5	0.6401±0.004	60.5±0.3	0.0412±0.001	-	0.0412±0.001	97.4±0.22
48	1.7600±0.012	41.2±0.1	1.7653±0.015	41.2±0.1	0.8510±0.0015	51.7±0.6	0.6563±0.005	69.7±0.3	0.0418±0.002	-	0.0418±0.002	97.7±0.25

% Inh = Inhibition; O.D. = Optical density at 660 nm

TABLE 7: ANTIMICROBIAL ACTIVITY OF POLYMER BOUND AMOXYCILLIN AS A FUNCTION OF TIME AND INCREASING MA CONTENT AGAINST *ST. AUREUS*

Time (h)	Control O.D.		Copoly-mer O.D.		P (BMA-MA-1) AMOX		P (BMA-MA-2) AMOX		P (BMA-MA-3) AMOX		Amoxycillin	
	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh	O.D.	% Inh
0	0.0040±0.00	-	0.0030±0.00	-	0.0040±0.00	-	0.0030±0.00	-	0.0020±0.00	-	0.0020±0.00	-
8	0.2710±0.07	12.6±0.1	0.2714±0.010	12.6±0.1	0.228±0.004	15.9±0.5	0.1591±0.003	43.3±0.5	0.0024±0.00	-	0.0024±0.00	99.1±0.75
16	0.4621±0.004	13.3±0.1	0.4622±0.002	13.3±0.1	0.3694±0.002	20.1±0.1	0.2374±0.002	48.7±1.0	0.0065±0.0001	-	0.0065±0.0001	98.6±0.30
24	0.7781±0.002	21.5±0.2	0.7773±0.004	21.5±0.2	0.5812±0.011	25.3±1.1	0.3971±0.006	49.0±1.0	0.0147±0.001	-	0.0147±0.001	98.1±0.12
32	0.8942±0.004	23.7±0.2	0.8960±0.003	23.7±0.2	0.6421±0.002	28.2±1.2	0.4102±0.001	54.1±0.75	0.0197±0.002	-	0.0197±0.002	97.8±0.22
40	1.1913±0.001	28.5±0.3	1.1911±0.001	28.5±0.3	0.8093±0.015	32.1±0.8	0.4563±0.003	61.7±0.4	0.0214±0.002	-	0.0214±0.002	98.2±0.25
48	1.4482±0.002	40.1±0.1	1.4486±0.002	40.1±0.1	0.8110±0.015	44.0±1.0	0.5018±0.002	65.4±0.4	0.0261±0.001	-	0.0261±0.001	98.2±0.30

% Inh = Inhibition; O.D. = Optical density at 660 nm

density of the medium increases. It may be noted that dead bacteria contributes to optical density. Data in Tables 5 to 7 clearly indicate that after initial induction period, the antimicrobial activity is observed by constant optical density in all cases, which is attributed by dead bacteria. Further the results were confirmed by withdrawing samples from each of the systems at time intervals indicated in Tables 5 to 7 and plating on nutrient agar medium. Initial and final colony counts were performed (results not provided). Observations showed the activity profile in the form: P(BMA-MA-3) > P(BMA-MA-2) > P(BMA-MA-1) > Pure polymer. In case of unbound amoxicillin low induction period as well as high inhibition (low optical density) was observed.

For various polymers bound amoxicillin (AMOX) samples, antimicrobial activity was observed in all the cases and found to increase with increase in maleic anhydride content in polymeric system. Table 5 to 7 shows antimicrobial activity of polymer bound amoxicillin as a function of time and increase in MA content towards each bacterial strain. P(BMA-MA-1, 2 and 3) showed maximum inhibition of 40%, 59.3% and 72.8% towards *Bacillus subtilis*, 40%, 44% and 65.4% against *Staphylococcus aureus* and 41.2%, 51.7% and 69.1% against *Escherichia coli* at 48 h of incubation.

This study provides a concept of charging a therapeutic level of active agent in the target site for a long duration and permits to manipulate the pharmacokinetic behavior of a drug. As a matter of fact, the bound drug is wholly released after 7 to 9 d as against the time of 30 min required for the unbound drug. This evidences its retarding action is due to the binding of the drug with the polymer matrix. The release behavior of drug depends on the kind of polymer system and the characteristics of the drug i.e. molecular weight and solubility. The release study clearly indicates that the amount of amoxicillin should be released in the range of minimum inhibitory concentration of the drug. In view of this the copolymer system with higher anhydride content are most suitable for releasing drug equivalent to MIC at constant rate for an extended period i.e. 6

to 9 d at a particular pH.

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